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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/195 — A1

(11) International Publication Number: **WO 99/37296**

(43) International Publication Date: 29 July 1999 (29.07.99)

(21) International Application Number: PCT/US99/01290

(22) International Filing Date: 22 January 1999 (22.01.99)

(30) Priority Data:
60/072,397 23 January 1998 (23.01.98) US

(71) Applicant: WARNER–LAMBERT COMPANY [US/US]; 201 Tabor Road, Morris Plains, NJ 07950 (US).

(72) Inventors: MAGNUS–MILLER, Leslie; 68 Rocklegde Drive, Livingston, NJ 07039 (US). SEGAL, Catherine, A.; 60 Shawnee Avenue, Rockaway, NJ 07866 (US).

(74) Agents: RYAN, M., Andrea; Warner–Lambert Company, 201 Tabor Road, Morris Plains, NJ 07950 (US) et al.

(81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HR, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GABAPENTIN AND ITS DERIVATIVES FOR THE TREATMENT OF MUSCULAR AND SKELETAL PAIN

(57) Abstract

The instant invention is a method of using certain analogs of glutamic acid and gamma–aminobutyric acid to relieve muscular/skeletal back pain.

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GABAPENTIN AND ITS DERIVATIVES FOR THE TREATMENT OF MUSCULAR AND SKELETAL PAIN

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The present invention relates to the use of analogs of glutamic acid and gamma-aminobutyric acid (GABA) for the treatment of muscular and skeletal pain. More particularly, the invention relates to the use of gabapentin for the treatment of non-neuropathic muscular and skeletal pain.

2. Description of Related Art

The compounds of the invention are known agents useful in antiseizure therapy for central nervous system disorders such as epilepsy, Huntington's chorea, cerebral ischemia, Parkinson's disease, tardive dyskinesia, and spasticity. It has also been suggested that the compounds can be used as antidepressants, anxiolytics, and antipsychotics. See WO 92/09560 (United States Serial Number 618,692 filed November 27, 1990) and WP 93/23383 (United States Serial Number 886,080 filed May 20, 1992).

WO 97/33858 teaches that compounds related to gabapentin are useful or treating epilespy, faintness attacks, hypokinesia, cranial disorders, neurodegenerative disorders, depression, anxiety, panic, pain, and neuropathological disorders. WO 97/33858 does not specific what forms of pain are treated.

Additionally, the compounds of the invention are known for treatment of neuropathic pain. For example, see Rosner H; Rubin L; Kestenbaum A., Gabapentin adjunctive therapy in neuropathic pain states. Clin J Pain, 1996 Mar, 12:1, 56-8; Segal AZ; Rordorf G., Gabapentin as a novel treatment for postherpetic neuralgia. Neurology, 1996 Apr, 46:4, 1175-6; Wetzel CH; Connelly JF., Use of gabapentin in pain management. Ann Pharmacother, 1997 Sep, 31:9, 1082-3; Zapp JJ., Postpoliomyelitis pain treated with gabapentin [letter]. Am Fam

Physician, 1996 Jun, 53:8, 2442, 2445; Cheville A, et al., Neuropathic pain in radiation myelopathy: a case report. Program book, American Pain Society (14th Annual Scientific Meeting). Abstract #95823, p. A-115; Sist T; Filadora V; Miner M; Lema M., Gabapentin for idiopathic trigeminal neuralgia: report of two cases. Neurology, 1997 May, 48:5, 1467; Waldman SD, Tutorial 28: Evaluation and Treatment of Trigeminal Neuralgia. Pain Digest (1997) 7:21-24; Mellick LB; Mellick GA., Successful treatment of reflex sympathetic dystrophy with gabapentin [letter]. Am J Emerg Med, 1995 Jan, 13:1, 96; Mellick GA; Seng MI., The use of gabapentin in the treatment of reflex sympathetic dystrophy and a phobic disorder. Am J Pain Manage 1995; 5:7-9; Mellick GA; Mellicy LB; Mellick LB., Gabapentin in the management of reflex sympathetic dystrophy [letter]. J Pain Symptom Manage, 1995 May, 10:4, 265-6; Mellick GA; Mellick LB., Reflex sympathetic dystrophy treated with gabapentin. Arch Phys Med Rehabil, 1997 Jan, 78:1, 98-105 and Mackin GA., Medical and pharmacologic management of upper extremity neuropathic pain syndromes. J Hand Ther, 1997 Apr-Jun, 10:2, 96-109.

Rosenberg et al., The effect of gabapentin on neuropathic pain, Clin. J. Pain, 1997 Sept, 1393), pp. 251-3 discloses a retrospective review of patients that were treated with gabapentin. One of the groups of patients was reported to have low back pain. These patients did not report improved pain scores after using gapapentin.

Muscular and skeletal pain is caused by a variety of physical injuries or damage to the muscles, bones and tissues of the body. A particularly debilitating form of this pain is chronic lower back pain. Chronic lower back pain is a general condition of pain related to physical damage to the muscles (eg, muscle trauma or overexertion), bones (eg, osteoarthritis of the lumber spine) and/or tissues (eg, fibromyalgia) of the back. Low back pain can also result from inflammation of the axial skeleton (eg, ankylosing spondylitis), conditions that create physical pressure on the nerves and tissues surrounding the spine (eg, sciatica and degenerative disk disease), or conditions/treatments that directly damage nerve

tissue (eg, postpoliomyelitis and chronic progressive radiation myelopathy [CPRM]).

In contrast, neuropathic pain is chronic pain that can develop after alteration and/or injury to any level of the nervous system, peripheral or central. The pain is typically burning and is associated with pressure on a nerve and/or actual damage to nervous tissue. Some types of chronic low back pain can have a neuropathic component (eg, sciatica, postpoliomyelitis and CPRM). Neuropathic conditions that have been treated with gabapentin include: postherpetic neuralgia, postpoliomyelitis, CPRM, HIV-related neuropathy, trigeminal neuralgia, diabetic neuropathy and Reflex Sympathetic Dystrophy (RSD). Of these, the cases of postpoliomyelitis and CPRM had pain associated with the back. The main distinction between back pain that is neuropathy in nature, and muscular/skeletal lower back pain is that nerve damage itself is the cause of pain. In the other conditions, properly working nerves are detecting body tissue damage and pressure secondary to inflammation. The two neuropathic conditions that reported a low back pain component clearly had the formation of anomalous nerve tissue or remodelling of the nerve pathway as the cause of pain.

Musculoskeletal pain is the unpleasant experience created by disease or dysfunction in the muscles, connective tissues, or by the stretching of the natural coverings of bones and joints. This type of condition can occur even when the associated sensory nerve fibers, by which the nociceptive impulses are sent from muscular tissue to the brain, are fully normal. On the other hand, neuropathic pain is the sensation created when any combination of pathophysiological processes causes sensory nerves to function badly, or in a hyperexcitable manner. One of the notable characteristics of neuropathic pain is that it can create the sensation that the source of symptoms is within its associated tissue, even when the nonneural tissue is perfectly healthy.

In attempting to understand the pain of spinal and paraspinal disorders, such as that which occurs in syndromes related to the low back, there is often a temptation to separate causes of such suffering into musculoskeletal pain from

neuropathic pain. There are patients whose low back pain may be predominantly musculoskeletal or neuropathic. In other patients the resulting symptoms are due to some degree of dysfunction of both types.

Many patients who seek medical treatment for low back pain are often able to associate the onset of their symptoms to sudden mechanical trauma, such as a vehicular accident or more often a lifting or bending injury. The main reason for this is that the low back is more prone to abnormal or poorly coordinated movement than most other portions of the musculoskeletal system. In part this is due to the fact that the lumbar spine is a weak zones between two larger body regions. The general thickness of the chest and upper abdomen and the size of the pelvis and proximal lower extremities are physically greater and therefore less mobile. The only thing that supports the segment of the spinal column from the 12^{th} dorsal or thoracic vertebra to the sacrum is the paravertebral muscles and ligaments.

Adults who do not have adequate muscle tone or strength to support the spine, i.e. those in whom the paraspinal muscles are deconditioned, are unable to prevent the vertebrae or intervertebral discs from being bent, stretched or twisted out of place. During sudden or prolonged, intense spinal activity against weight or resistance, this weaker zone is susceptible to many different types of pain-producing mechanism.

For example, joints, including the spinal facets, may be twisted and become inflamed. Paraspinal muscles may be stretched to the point of having small penetrating vessels torn within the muscle tissue. Consequently the tissue repair response may create the lying down of multiple small areas of scar tissue within the muscle, which can stimulate further pain symptoms during contraction.

Similarly ligaments may be stretched and their sites of attachment may be torn away leaving microscopic hemorrhages and spinal instability.

Disc materials may be twisted in a manner where undue pressure from

above causing an actual internal shift of the nuclear material, resulting in a bulging or distortion of the shape of the disc. Despite longstanding opinions that disc capsules do not have adequate innervation to produce clinical pain when they are mechanically displaced, it has been confirmed that the posterior aspects of discs do indeed have innervation which creates pain when the disc capsule (annulus fibrosis) is stretched. Severe or sudden mechanical force can cause its jelly-like contents (nucleus pulpous) to shift and create pressure gradients against the inside wall of the disc capsule (annulus fibrosis) great enough for the disc to rupture.

Once the disc material is extruded outside its usual boundaries, it can put pressure on the nerve roots that are attempting to exit from the spinal canal. If enough of the disc material is ruptured or protruding, it may place true mechanical compression on the blood vessels which provide nutrition and oxygen to the nerve (versa nervure). The resulting schema will then make the nerve root dysfunctional, creating a specific form of neuropathic pain known as radiculopathy.

Because the pathological conditions that create radicular pain are based on a direct involvement of nerves, and not just a set of messages that have been detected by healthy nerve endings in painful muscle, this type of pain is better classified as neuropathic than musculoskeletal.

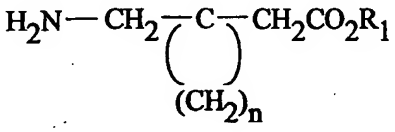
Reflex Sympathetic Dystrophy (RSD) is a form of neuropathic pain that persists long after the cessation of cellular damage or healing of a physical injury. It is characterized by a severe burning pain secondary to autonomic (increased sympathetic activity) and/or dystrophic (skin and bone atrophy, increased hair and nail growth, cellular fibrosis, and possible alterations in cellular norepinephrine and sympathetic receptor sensitivity) changes in the tissue of the affected region. Such pain in the absence of any ongoing tissue damage along with the autonomic and/or dystrophic changes are distinguishing characteristics from conditions causing general low back pain. Moreover, the continued pain of RSD is typically out of proportion to the initial tissue damage and generally affects an extremity (eg, right shoulder and arm).

The current treatments for lower back pain include exercise, spinal manipulation, bed rest, traction and/or drug therapy. Drugs used to treat lower back pain include acetaminophen, tizanidine, mefenamic acid, chlormethoxazone, -paracetamol, ethoheptazine-aspirin-meprobamate, piroxicam, diflunisal, naproxen sodium, tricyclic antidepressants, indomethacin, cyclobenzaprine, baclofen and ibuprofen.

Until the present invention, there has not been any report of using gabapentin, which was originally used for treating the neurological disorder epilepsy, and has been further used to treat neurologically based pain (neuropathic pain), to treat muscular/skeletal based pain, such as chronic lower back pain. Nor has gabapentin been studied in low back pain conditions where the nervous system is functioning normally.

SUMMARY OF THE INVENTION

This invention provides a method for treating muscular and skeletal pain comprising administering to a subject suffering from such pain an effective amount of a GABA analog. A preferred embodiment utilizes a cyclic amino acid compound of Formula I

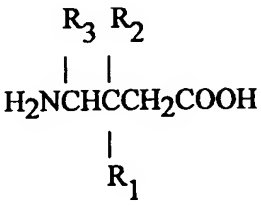


I

wherein R_1 is hydrogen or lower alkyl and n is an integer of from 4 to 6, and the pharmaceutically acceptable salts thereof. An especially preferred embodiment utilizes a compound of Formula I where R_1 is hydrogen and n is 4, which compound is 1-(aminomethyl)-cyclohexane acetic acid, known generically as gabapentin.

In another embodiment, the invention includes treating muscular and skeletal pain with a compound of Formula II.

Formula II



II

or a pharmaceutically acceptable salt thereof wherein

R_1 is a straight or branched alkyl of from 1 to 6 carbon atoms, phenyl, or cycloalkyl of from 3 to 6 carbon atoms;

R_2 is hydrogen or methyl; and

R_3 is hydrogen, methyl, or carboxyl.

Preferred compounds of the invention are those wherein R_3 and R_2 are hydrogen, and R_1 is -$(CH_2)_{0-2}$-i C_4H_9 as an (R), (S), or (R,S) isomer.

The more preferred compounds of Formula II invention are (S)-3-(aminomethyl)-5-methylhexanoic acid and 3-aminomethyl-5-methyl-hexanoic acid, now known generically as pregabalin.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The method of this invention utilizes any GABA analog. A GABA analog is any compound derived from or based upon gamma-aminobutyric acid. The compounds are readily available, either commercially, or by synthetic methodology well known to those skilled in the art of organic chemistry. The preferred GABA analogs to be utilized in the method of this invention are cyclic amino acids of Formula I. These are described in U.S. Patent 4,024,175, which is incorporated herein by reference. Another preferred method utilizes the GABA analogs of Formula II, and these are described in U.S. Patent 5,563,175, which is

incorporated herein by reference.

All that is required to practice the method of this invention is to administer a GABA analog in an amount that is effective to treat the muscular and/or skeletal pain. Such anti-pain amount will generally be from about 1 to about 300 mg per kg of subject body weight. Typical doses will be from about 10 to about 5000 mg per day for an adult subject of normal weight. It is expected that common doses that might be administered for muscular and skeletal pain could be from 100 mg. Three times a day up to 600 mg. Four times a day. Commercially available capsules of 100 mg, 300 mg, 600 mg and 800 mg of gabapentin can be administered. Alternate forms include liquids and commercially available film-coated tablets.

The compounds of the present invention may form pharmaceutically acceptable salts with both organic and inorganic acids or bases. For example, the acid addition salts of the basic compounds are prepared either by dissolving the free base in aqueous or aqueous alcohol solution or other suitable solvents containing the appropriate acid and isolating the salt by evaporating the solution. Examples of pharmaceutically acceptable salts are hydrochlorides, hydrobromides, hydrosulfates, etc. as well as sodium, potassium, and magnesium, etc. salts.

The compounds of the Formula II can contain one or several asymmetric carbon atoms. The invention includes the individual diastereomers or enantiomers, and the mixtures thereof. The individual diastereomers or enantiomers may be prepared or isolated by methods already well known in the art.

Formulating the active compound in dosage unit form with a pharmaceutical carrier produces pharmaceutical compositions useful in the present invention. Some examples of dosage unit forms are tablets, capsules, pills, powders, aqueous and nonaqueous oral solutions and suspensions, and parenteral solutions packaged in containers containing either one or some larger number of dosage units and capable of being subdivided into individual doses.

Some examples of suitable pharmaceutical carriers, including pharmaceutical diluents, are gelatin capsules; sugars such as lactose and sucrose; starches such as corn starch and potato starch, cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, methyl cellulose, and cellulose acetate phthalate; gelatin; talc; stearic acid; magnesium stearate; vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, and oil of theobroma; propylene glycol, glycerin; sorbitol; polyethylene glycol; water; agar; alginic acid; isotonic saline, and phosphate buffer solutions; as well as other compatible substances normally used in pharmaceutical formulations. The compositions of the invention can also contain other components such as coloring agents, flavoring agents, and/or preservatives. These materials, if present, are usually used in relatively small amounts. The compositions can, if desired, also contain other therapeutic agents.

The percentage of the active ingredients in the foregoing compositions can be varied within wide limits, but for practical purposes it is preferably present in a concentration of at least 10% in a solid composition and at least 2% in a primary liquid composition. The most satisfactory compositions are those in which a much higher proportion of the active ingredient is present.

Routes of administration of the subject compound or its salts are oral or parenteral. For example, a useful intravenous dose is between 5 and 50 mg and a useful oral dosage is between 20 and 800 mg. The dosage is within the dosing range used in treatment of pain or as would be with the needs of the patient as described by the physician.

A unit dosage form of the GABA analog to be used in this invention may also comprise other compounds useful in the treatment of pain.

The advantages of using the compounds of Formula I and II, especially gabapentin and pregabalin, in the present invention include the relatively nontoxic nature of the compounds, the ease of preparation and the fact that the compounds

are well-tolerated. Gabapentin has few interactions with major classes of drugs since it is not metabolized in the liver, but rather excreted unchanged from the body. Further, the drugs are not metabolized in the body. The subjects treated with the method of the present invention are mammals, including humans.

Example 1

A 53-year-old man presented with a long history of back pain. His initial back injury resulted from a fall while installing a valve. Over the next few years he reinjured his back several times. The third injury caused significant disability and pain that did not respond to medications. As a result, surgery was performed four years ago and steel rods and hangers were inserted. Despite the surgery and subsequent rehabilitation, he had significant pain and disability. He could not stand straight and could not maintain any position (sitting, standing, or walking) for any length of time. He was maintained on an opiate pain relief regimen of transdermal Fentanyl patches and six Vicodin tablets per day but felt constant pain.

One year ago, his physician arranged for a trial of gabapentin. Starting with an initial dose of one 100 mg. Capsule three times a day, he began to experience pain relief within a few hours. He commented to his wife that for the first time in years he felt no pain. By the end of the second day, he felt well enough to go out socially. He went to a wedding where he felt good enough to dance for the first time in years. One year later, he is maintained on a regimen of gabapentin 300 mg. three times a day and the Fentanyl patch. Vicodin is available on an as needed basis, but he said he needs fewer tablets. Unlike before the gabapentin trial, he can stand straight and can walk again. The pain relief in his back condition has been sustained for over one year.

Example 2

This 50 year old Caucasian female patient was referred for diagnosis and management of her chief complaint of aching and fatigue and persistent muscle

pain on a daily basis, with secondary or comorbid complaints of her legs jerking at night, as well as an intermittent band like pain around her head.

The history of the presenting illness, relative to the chief complaint, included the following symptom characteristics: Onset was gradual, beginning approximately 17 years ago, without any clear-cut relationship to trauma. The regional distribution of the current symptoms included pain that involves multiple areas and is generally bilateral and symmetric. This pain involved the trapezius muscles of the neck, anterior shoulders and scapulae, clavicles, lower costal margins, hips and buttocks, elbows, wrists and medial aspects of the knees. Visual analysis of the Pain Drawing instrument completed by the patient revealed no clear-cut radicular or articular patterns. Pain occurred simultaneously in all areas, without clearly defined radiation or referred pain. The patient described the quality of the multi-regional pain as aching, with independent burning pain in the feet and legs occurring only at night. As mentioned above, the headache was described as band-like. The timing was continuous, with frequent variations in severity. At the initial visit, the pain intensity of most areas was reported, using a standard Numeric Rating Scale (NRS-11, with zero being no pain and 10 representing the worst pain imaginable) as 4-6. The pain was made worse with exercise, during cold weather and generally increased at night. No specific agents or activities made the pain any better. Other symptoms included tingling of both feet.

Past medical history was positive for the usual childhood illnesses, but otherwise negative. Family history was positive for arthritis, heart disease and hypertension. Social history was negative for tobacco or alcohol. The allergy history was positive for codeine, meperidine (Demerol), carisoprodol (Soma) and penicillin.

Physical Examination

The patient's weight was 150 pounds, height was 5'1". Vital signs at the initial visit were: Pulse-68 per minute, BP (Right arm sitting)-122/70,

Respirations- 12 and p.o. Temperature- 97.2 F.

General examination revealed that Head, eyes, ears, nose and throat were normal. Heart sounds were normal. Chest was clear. Abdomen revealed no acute abnormalities. Spinal examination revealed that active range of motion was reduced in the cervical spine. Paraspinal muscle spasm and tender points were noted at multiple sites during palpation. Straight leg raising produced pain at 70 degrees on the left, and at 80 degrees on the right.

Neurological examination revealed mental status changes (mild depression) which seemed to be appropriate for chronic pain. Speech was normal. Cranial nerves, including fundi, were normal. Upper extremity bulk, power, tone and range of motion were normal. Power was 4/5-5/5 in most muscle groups, but pain and soreness were noted by the patient. Reflexes were normal in both upper and lower extremities. Plantar reflexes were normal. Sensory examination was normal to cold, pin, and vibration. Stance and gait were within normal limits.

Impression / Differential Diagnosis

Myofascial pain syndrome versus fibromyalgia syndrome, paraspinal muscle spasm, cervical radiculopathy, lumbar radiculopathy.

Diagnostic Test Results

Needle EMGs of the upper extremities revealed moderate C5-6 root irritation on the right, but lower extremity EMGs were normal. Nerve Conduction Velocity studies of both upper and both lower extremities were fully normal. MRI revealed degenerative changes at several levels in the cervical spine with some resulting foraminal narrowing on the right at C5-6, but no herniated intervertebral discs and no spinal stenosis. On the sagittal views there was straightening of the normal lordotic curve consistent with spasm.

General Classification of Pain Syndrome

Primarily musculoskeletal pain (Myofascial pain syndrome vs. fibromyalgia) with radiographic evidence for degenerative changes of the cervical spine, with secondary independent root irritation created by the associated neuroforaminal narrowing.

Treatment

The patient had previously been unresponsive to OTC preparations such as acetaminophen and ibuprofen, and had been allergic to codeine, meperidine and carisoprodol. While diagnostic tests were being conducted she was provided a therapeutic trial of tizanidine hcl (Zanaflex) for her painful muscle spasms. This was titrated upward over several days from 1 mg three times a day to 4 mg three times a day. At her follow-up appointment six weeks later she stated that she had noticed some improvement (up to 25% pain reduction)in her myofascial symptoms, but she could not tolerate the usual dose due to significant drowsiness. She therefore had to reduce the tizanidine hcl to 1 mg at breakfast and lunch and 4 mg at bedtime, which reduced the effectiveness of the treatment.

Her various allergies to medication were discussed, as well as the benefit of avoidance of narcotic analgesics for chronic pain of this type. After an explanation of the adjuvant, (not indicated) use of antiepileptic drugs for some forms of neuropathic pain, and potential side effects, she was then offered a therapeutic trial of gabapentin (Neurontin). This was added on an ascending schedule, with increasing steps as follows: Day 1 and 2, 100 mg four times a day (with meals and at bedtime), Day 3 and 4, 200 mg four times a day, Day 5 and 6 300 mg four times a day, and thereafter 300 mg with each meal and 600 mg at bedtime. At her follow-up visit three weeks later she described her generalized aching and fatigue were slightly improved and she noted that she had a reduced number of what she described as "knotted up tender spots" in her back. However she had admitted that she had only taken one capsules of gabapentin at night, not two. She was instructed to increase her total daily dose by 25 % (300 mg) to a

total daily dose of 1500 mg.

During that time she was seen for neurosurgical consultation regarding any possible corrective measures that might be directed toward the potential for neuroforaminal stenosis. He suggested cervical traction but she discontinued this after one or two sessions due to increased pain. At the time of his follow up visit, after the gabapentin 1500 mg/day had been added, the neurosurgeon's report indicated "she has improved significantly..her neck seems to have improved even more than the lower back..since she has improved to this degree she would like to maintain the current course of action".

She was reassessed in our clinic two weeks later and found to have a reduction of the total number of painful regions from 25 down to 6, and her general pain intensity had gone from 4-6, down to 1-2, with only an occasional site with pain of 4. She describes her general clinical response as "50 per cent improved" since her original visit. She was offered the option of increasing the gabapentin to 1800 mg/day by adding one 300 mg capsule at night. Thus far she has had no side effects from the addition or increasing scale of the gabapentin.

Example 3

This 67 year old Caucasian female patient was referred for diagnosis and management of her chief complaint of multiregional pain involving intrascapular, thoracic and lumbar areas, with secondary or comorbid complaints of generalized weakness, as well as right sided neck pain and upper extremity paresthesias.

The history of the presenting illness, relative to the chief complaint, included the following symptom characteristics: Onset was gradual, beginning approximately 10 years ago for the low back pain and 3-4 years ago for the other regional pain symptoms. There was no relationship to trauma. The regional distribution of the current symptoms included pain that involves multiple areas and was generally bilateral and symmetric except for the upper extremity pain that was greater on the right side. The pain involved the trapezius muscles of the neck,

shoulders and scapulae, mid thoracic area, sacrum, knees and heels. Visual analysis of the Pain Drawing instrument completed by the patient revealed no clear-cut radicular or articular patterns. Pain occurred simultaneously in all areas, without clearly defined radiation or referred pain. The patient described the quality of the multiregional pain as aching, with only occasional shooting pain. She also stated that many of the painful areas felt "swollen". At the initial visit, the pain intensity of most areas was reported, using a standard Numeric Rating Scale (NRS-11, with zero being no pain and 10 representing the worst pain imaginable) as 4-8 (average=6). The timing of the pain was continuous, with the intensity made worse with any kind of physical activity. No specific agents or reduction of activities made the pain any better. Other symptoms included unsteadiness while walking and a painful (antalgic) gait pattern.

Past medical history was positive for the usual childhood illnesses, headaches, hypertension and arthritis. Family history was positive for arthritis, heart disease and hypertension. Social history was negative for alcohol but the patient does smoke one ppd. The allergy history was positive for morphine, meperidine (Demerol), tetracycline and "all arthritis medications".

Physical Examination

The patient's weight was 170 pounds, height was 5'5". Vital signs at the initial visit were: Pulse-64 per minute, BP (Right arm sitting)-148/88, Respirations- 16 and p.o. Temperature- 98.5 F

General examination revealed that Head, eyes, ears, nose and throat were normal. Heart sounds S1 and S2 were normal but there was a low-grade (III/VI) systolic murmur. Chest was clear. Abdomen revealed old hysterectomy scar. Spinal examination revealed that active range of motion was only minimally reduced in the cervical spine. Paraspinal muscle spasm and tender points were noted at multiple sites during palpation. Straight leg raising was performed to 85 degrees bilaterally.

Neurological examination revealed mental status changes (mild depression) which seemed to be appropriate for chronic pain. Speech was normal. Cranial nerves, including fundi, were normal. Upper extremity bulk, power, tone and range of motion were normal. Power was 4/5-5/5 in most muscle groups, but pain and soreness were noted by the patient. Reflexes were mildly reduced in both upper extremities, but patellars were reduced and achilles reflexes were absent bilaterally. Plantar reflexes were normal. Sensory examination revealed local hyperesthesia to touch and light palpation but was within normal limits to cold and pin. Vibration was mildly reduced bilaterally below the ankles. Stance and gait were within normal limits.

Impression / Differential Diagnosis

Myofascial pain syndrome versus fibromyalgia syndrome, paraspinal muscle spasm, cervical radiculopathy, lumbar radiculopathy.

Diagnostic Test Results

Needle EMGs of the upper extremities revealed mild C5-6 root irritation on the left and mild C3-4 irritation on the right, but lower extremity EMGs were normal. Nerve Conduction Velocity studies of both upper and both lower extremities were fully normal. MRI revealed prominent bulging discs at C4-5 and C5-6, and moderately bulging discs at C3-4 and C6-7, but no disc herniation or spinal stenosis.

General Classification of Pain Syndrome

Primarily musculoskeletal pain (Myofascial pain syndrome vs. fibromyalgia) with radiographic evidence for changes of the cervical spine associated with bulging intervertebral discs.

Treatment

The patient had previously been unresponsive to OTC preparations such as acetaminophen, and had not had any significant benefit from codeine containing compounds (Tylenol # 3, four times a day). She had been taking Robaxin 750 mg twice a day, which was tapered and discontinued due to its ineffectiveness. While diagnostic tests were being conducted she was provided a therapeutic trial of tizanidine Hcl (Zanaflex) for her aching and for painful muscle spasms. This was initiated at a low dose (1mg three times a day) due to the patient's concerns about drowsiness. At her follow-up appointment six weeks later she stated that she had noticed some reduction in the number of painful areas and her pain intensity had decreased into the range of 2-5, with the average value of 3.5. She was instructed to titrate the tizanidine upward to 2 mg three times a day. Shortly thereafter she called to state that she "could not function" due to excessive sleepiness at 6 mg per day and she requested that this be reduced to 4 mg a day (2 mg twice a day). She experienced sleepiness and dry mouth with even this low dose and decreased the tizanidine Hcl to 2mg once a day. She returned five weeks later stating that there was no change in her symptoms, specifically regarding the intrascapular pain, the intensity of which had increased from 2 to 5.

Her various allergies to medication were discussed, as well as the benefit of avoidance of narcotic analgesics for chronic pain of this type. After an explanation of the adjuvant, (not indicated) use of antiepileptic drugs for some forms of neuropathic pain, and potential side effects, she was then offered a therapeutic trial of gabapentin (Neurontin). This was added on an ascending schedule, with increasing steps as follows: Day 1 and 2, 100 mg four times a day (with meals and at bedtime), Day 3 and 4, 200 mg four times a day, Day 5 and 6 300 mg four times a day, and thereafter 300 mg with each meal and 600 mg at bedtime. At her follow-up visit five weeks later she stated that her intrascapular pain had been reduced in frequency and severity, and her pain had decreased to zero. Her pain drawing instrument was essentially blank, i.e. no shaded areas indicating any spontaneous pain. She did, however, have some residual trigger points and tender points, which were only symptomatic during direct palpation, and she chose to have these treated with trigger point injections during a

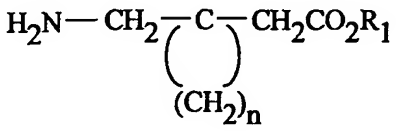
previously scheduled visit to an anesthesiologist. She was seen again in our clinic two months later, still on gabapentin (Neurontin) and she continued to be pain free. She had not had any further trigger point injections, was only taking 2mg tizanidine hcl at bedtime for rest, and had reduced her gabapentin to 1200 mg per day from 1500 mg per day. She was taking no narcotic analgesics, but continued on her antihypertensive medication and her hormone supplement that she had been taking since the first visit.

During the subsequent three months the patient indicated that, since she had reduced her gabapentin from 1500 mg a day to 1200 mg a day that she noticed some slight and transient return of her symptoms. She requested that she be permitted to increase it back to 1500 mg a day. She has since remained essentially asymptomatic on the previous schedule. Her personal and pharmacy records indicate that she is not taking any narcotic analgesics.

Although the invention has been described with reference to specific preferred embodiments, those skilled in the art will recognize that variations and modifications may be made that are within the spirit of the invention and within the scope of the claims.

What is claimed is:

1. A method for treating muscular and skeletal pain comprising administering a therapeutically effective amount of a compound of Formula I



I

wherein R_1 is hydrogen or lower alkyl and n is an integer of from 4 to 6, and the pharmaceutically acceptable salts thereof.

2. The method according to claim 1 wherein the muscular and skeletal pain is lower back pain.

3. The method according to Claim 1 wherein the compound is gabapentin.

4. The method according to Claim 2 wherein the compound is gabapentin.

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 99/01290

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	B. EPSTEIN ET AL.: "The Use of Gabapentin for Neuropathic and Musculoskeletal Pain: A Case Series" JOURNAL OF NEUROLOGIC REHABILITATION, vol. 12, no. 2, September 1998, pages 81-86, XP002101736 see the whole document	1-4
P,X	WO 98 46601 A (WHITESITT CELIA ANN ;LILLY CO ELI (US); SHANNON HARLAN EDGAR (US)) 22 October 1998 see abstract see page 4, last paragraph see page 7, last paragraph - page 8, paragraph 1; claims 1-5,14	1-4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 May 1999	18/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoff, P

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 99/01290

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 58641 A (TAYLOR CHARLES PRICE JR ;WARNER LAMBERT CO (US); WESTLUND HIGH KAR) 30 December 1998 see abstract see page 1, line 1 - page 5, line 27; claims; example 4	1,3
P,X	WO 98 19674 A (BENDTSEN LARS ;JENSEN RIGMOR (DK); MADSEN ULF (DK); OLESEN JES (DK) 14 May 1998 see abstract; claims; examples 1,6	1,3
X	"Postpoliomyelitis pain treated with gabapentin." AMERICAN FAMILY PHYSICIAN, (1996) 53/8 (2442-2443). ISSN: 0002-838X CODEN: AFPYAE, XP002101737 United States cited in the application see the whole document	1-4
X	MELLICK G A ET AL: "REFLEX SYMPATHETIC DYSTROPHY TREATED WITH GABAPENTIN" ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION, vol. 78, no. 1, January 1997, pages 98-105, XP002043784 cited in the application see the whole document	1-4
X	MELLICK G A ET AL: "GABAPENTIN IN THE MANAGEMENT OF REFLEX SYMPATHETIC DYSTROPHY" JOURNAL OF PAIN AND SYMPTOM MANAGEMENT, vol. 10, no. 4, May 1995, page 265/266 XP002043783 cited in the application see the whole document	1,3
X	ROSENBERG J M ET AL: "The effect of gabapentin on neuropathic pain." CLINICAL JOURNAL OF PAIN, (1997 SEP) 13 (3) 251-5. JOURNAL CODE: BEG. ISSN: 0749-8047., XP002101738 United States cited in the application see the whole document	1,3
X	WETZEL C H ET AL: "Use of gabapentin in pain management." ANNALS OF PHARMACOTHERAPY, (1997 SEP) 31 (9) 1082-3. REF: 8 JOURNAL CODE: BBX. ISSN: 1060-0280., XP002101739 United States see the whole document	1,3

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/01290

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MUELLER M E ET AL: "Gabapentin for relief of upper motor neuron symptoms in multiple sclerosis." ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION, (1997 MAY) 78 (5) 521-4 JOURNAL CODE: 8BK. ISSN: 0003-9993., XP002101740 United States see the whole document	1,3
X	GB 1 465 229 A (WARNER LAMBERT CO) 23 February 1977 see the whole document	1,3
X	FIELD M J ET AL: "Evaluation of gabapentin and S-(+)-3-isobutylgaba in a rat model of postoperative pain." JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1997 SEP) 282 (3) 1242-6. JOURNAL CODE: JP3. ISSN: 0022-3565., XP002101741 United States see the whole document	1,3
A	SHIMOYAMA, NAOHITO ET AL: "Spinal gabapentin is antinociceptive in the rat formalin test" NEUROSCI. LETT. (1997), 222(1), 65-67 CODEN: NELED5;ISSN: 0304-3940,1997, XP002101742 see the whole document	1-4
A	FIELD M J ET AL: "GABAPENTIN (NEURONTIN) AND S-(+)-3-ISOBUTYLGABA REPRESENT A NOVEL CLASS OF SELECTIVE ANTIHYPERALGESIC AGENTS" BRITISH JOURNAL OF PHARMACOLOGY, vol. 121, no. 8, 1997, pages 1513-1522, XP002043785 see the whole document	1-4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/01290

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-4
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-4 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern ..al Application No
PCT/US 99/01290

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9846601 A	22-10-1998	AU 7247898 A	11-11-1998
WO 9858641 A	30-12-1998	AU 8375898 A	04-01-1999
		HR 980342 A	28-02-1999
WO 9819674 A	14-05-1998	AU 4863297 A	29-05-1998
GB 1465229 A	23-02-1977	DE 2460891 A	01-07-1976
		AT 340892 B	10-01-1978
		AT 975075 A	15-05-1977
		AU 8774175 A	23-06-1977
		BE 836835 A	18-06-1976
		CA 1052811 A	17-04-1979
		CH 612665 A	15-08-1979
		CH 612666 A	15-08-1979
		CH 612664 A	15-08-1979
		DE 2543821 A	14-04-1977
		DK 581475 A,B,	22-01-1976
		FI 753613 A,B,	22-06-1976
		FR 2294697 A	16-07-1976
		IE 42382 B	30-07-1980
		JP 941538 C	20-02-1979
		JP 51088940 A	04-08-1976
		JP 53024064 B	18-07-1978
		LU 74058 A	20-07-1976
		NL 7514900 A,B,	23-06-1976
		SE 423385 B	03-05-1982
		SE 7514442 A	22-06-1976
		US 4024175 A	17-05-1977
		US 4087544 A	02-05-1978

Cellular and Molecular Neurobiology, Vol. 22, No. 2, April 2002 (© 2002)

Down-Regulation of N-Type Voltage-Activated Ca^{2+} Channels by Gabapentin

Alfonso Vega-Hernández[1] and Ricardo Felix[1,2]

Received March 18, 2002; accepted May 2, 2002

SUMMARY

1. Although the cellular and molecular mechanisms of the anticonvulsant action of gabapentin (GBP) remain incompletely described, in vitro studies have shown that GBP binds to the $\alpha_2\delta$ subunit of the high voltage-activated (HVA) Ca^{2+} channels.

2. In this report, we analyzed the effects of GBP on the functional expression of HVA Ca^{2+} channels in the PC12 cell line model system. Negligible inhibition of Ca^{2+} channel activity was observed after acute treatment, but a significant decrease in Ca^{2+}current amplitude was promoted by chronic exposure to GBP.

3. Consistent with this, radioligand binding experiments showed a comparable reduction in the total number of membrane HVA N-type channels after GBP treatment.

KEY WORDS: Ca^{2+} channels; gabapentin; ω-conotoxin GVIA; PC12 cells.

INTRODUCTION

Gabapentin (GBP; 1-(aminomethyl) cyclohexaneacetic acid) is a cyclic γ-aminobutyric acid (GABA) analog that has been shown to have anticonvulsant activity in a variety of animal seizure models and is also effective in the treatment of human partial and generalized tonic–clonic seizures (McLean, 1995). In spite of its demonstrated efficacy, the anticonvulsant mechanism of GBP is not known. Although its close structural similarity to GABA, it has been suggested that GBP does not bind to GABA receptors, and does not affect the uptake or the degradation of this inhibitory neurotransmitter (Taylor *et al.*, 1998). In vitro studies have shown also that GBP can modify the electrophysiological properties of central neurons in cell culture after prolonged (Wamil and McLean, 1994), but not brief (Rock *et al.*, 1993; Wamil and McLean, 1994) exposure at concentrations in the range used in clinical trials. However, GBP does not reduce Na^+ current through recombinant channels expressed in mammalian cells, suggesting that the effects on action potential firing rates could involve other types of ion channels (Taylor, 1993; Xie *et al.*, 2001). In line with

[1] Department of Physiology, Biophysics and Neuroscience, Cinvestav-IPN, Mexico City, Mexico.

[2] To whom correspondence should be addressed at Departamento de Fisiología, Biofísica y Neurociencias, Cinvestav-IPN, Avenida IPN # 2508, Colonia Zacatenco, CP 07000, México DF, México; e-mail: rfelix@fisio.cinvestav.mx.

0272-4340/02/0400-0185/0

this, a high affinity GBP-binding protein purified from brain membranes has been characterized. N-terminal sequencing identified the protein as the $\alpha_2\delta$ subunit of the HVA Ca^{2+} channels (Gee *et al.*, 1996; Wang *et al.*, 1999). This auxiliary subunit is a type I transmembrane protein associated to the Ca^{2+} channel pore-forming subunits, and consists of 2 NH_2-terminal signal sequence and a transmembrane domain near its carboxyl terminus (Gurnett *et al.*, 1996). Experimental evidence supports a role for the Ca^{2+} channel $\alpha_2\delta$ subunit in regulating channel surface expression and stabilization (Felix, 1999). Interestingly, transient transfection of the $\alpha_2\delta$ subunit cDNA in COS-7 cells increased significantly binding of [^{3}H]-GBP, consistent with the expression of the $\alpha_2\delta$ protein, as measured by Western blotting (Gee *et al.*, 1996; Marais *et al.*, 2001). Lastly, recent functional studies have shown that HVA Ca^{2+} channels, predominantly of the L-type, can be directly affected by GBP (Alden and Garcia, 2001; Stefani *et al.*, 1998, 2001). In this report we show what is to our knowledge the first evidence for a long-term down-regulation in the expression of HVA Ca^{2+} channels predominantly of the N-type, which in addition to the demonstrated modulation of L-type channel activity, might be important to the antiepileptic action of GBP.

MATERIALS AND METHODS

Cell Culture and Materials

The rat pheochromocytoma PC12 cell line was obtained from the American Type Culture Collection and was grown in standard conditions (Liu *et al.*, 1996). Culture medium was replenished every day. GBP, a generous gift from Dr Kevin P. Campbell (University of Iowa), was dissolved in sterile distilled water (~2.9 mM stock solution) and diluted to the desired concentration (30 μM) in the culture medium. All other chemicals were of reagent grade.

Electrophysiology

Undifferentiated PC12 cells were subjected to the standard whole-cell patch-clamp technique as described previously (Liu *et al.*, 1996). Briefly, currents were recorded using an Axopatch 200 A amplifier and filtered at 1–2 kHz, digitized at 50 kHz and analyzed with pClamp software. The bath solution contained (in mM) 20 $BaCl_2$, 125 tetraethylammonium chloride, 10 HEPES, and 5 glucose (pH 7.3). The internal solution consisted of (mM) 130 CsCl, 2 $MgCl_2$, 11 EGTA, 20 HEPES, 2 Na_2ATP, 0.1 GTP, and 5 glucose (pH 7.3). Currents were obtained from a holding potential (HP) of −90 mV applying 50 or 150-ms test pulses every 30 s. Data were leak subtracted on line by a standard P/4 protocol. All experiments were performed at 20–22°C.

Radioligand Binding

Undifferentiated PC12 cell microsomes were prepared as detailed elsewhere (Liu *et al.*, 1996). In brief, cells were lifted off plates into PBS, collected by centrifugation, resuspended in lysis buffer in the presence of protease inhibitors and

homogenized. Aliquots (50 μg protein) of cell microsomes were resuspended in a total volume of 200 μL binding buffer (20 mM HEPES, pH 7.4, 0.1% BSA, 75 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, and protease inhibitors) and incubated with a saturating concentration of either [^{125}I]-ω-Conotoxin GVIA (ω-CTX) or [^{3}H]-L Glutamine (Gln) (0.75 and 100 nM, respectively) at room temperature. After 30 min the receptor–ligand complexes were collected and washed on Whatman GF/B filters using a cell harvester. Bound radioactivity was determined using liquid scintillation counting.

RESULTS AND DISCUSSION

Because the $\alpha_2\delta$ subunit of the HVA Ca^{2+} channels has shown to be the receptor for GBP (Gee *et al.*, 1996; Wang *et al.*, 1999), we investigated the possible functional repercussion of the GBP treatment on the whole-cell patch-clamp Ba^{2+} currents through Ca^{2+} channels in undifferentiated PC12 cells. Initial studies indicated that superfusion of the cells with GBP produced only marginal effects on the current recorded. Similarly, no effects on Ca^{2+} currents were observed after 12 and 24 h of GBP incubation (data not shown). In contrast, a significant inhibition of current density was observed upon chronic treatment (72 h) with 30 μM GBP (Fig. 1(A) and (B)). According to previous reports GBP acute block of Ca^{2+} currents in neurons is saturated at 25 μM (Sutton *et al.*, 2002). Although GBP chronic treatment markedly reduced current density, no evident changes in the voltage-dependence (Fig. 1(C)) and the waveform (Fig. 1(D)) of the currents were observed (see also Table I). Together these results suggest that GBP does not affect the function of the channels, but induces a significant reduction in the number of functional channels in the plasma membrane. To test this possibility, we performed radioligand binding experiments using ω-CTX, a selective inhibitor of neuronal N-type HVA Ca^{2+} channels, an important component of the whole-cell Ca^{2+} current in PC12 cells (Usowicz *et al.*, 1990). Figure 2(A) shows the comparison of ω-CTX binding to microsomes from undifferentiated PC12 cells, using a saturating concentration of the radiolabeled toxin. As can be seen, chronic treatment with GBP significantly decreased (~46%) specific binding. When compared to the ~41% inhibition in Ca^{2+} current density, this data suggests a correlation between GBP ability to inhibit current density and its ability to decrease ω-CTX binding.

These results could be explained by an extracellular interaction between the $\alpha_2\delta$ and GBP. This interaction could abolish the stabilizing effect of $\alpha_2\delta$ increasing the internalization of the channel complexes present in the plasma membrane. Alternatively, the results could be explained by an intracellular interaction between $\alpha_2\delta$ and GBP. In this case the drug could interact with the Ca^{2+} channel subunit in the interior of the cell disrupting (or preventing) the association of $\alpha_2\delta$ with the channel complex. This action would lead to a deficient trafficking of the channels to the plasma membrane. Because previous studies have shown that GBP is actively absorbed by the cells via the system L amino acid transporter, this idea could be indirectly tested performing specific Gln binding experiments in the absence and presence of GBP. As shown in Fig. 2(B) Gln binding is inhibited by saturating concentrations of

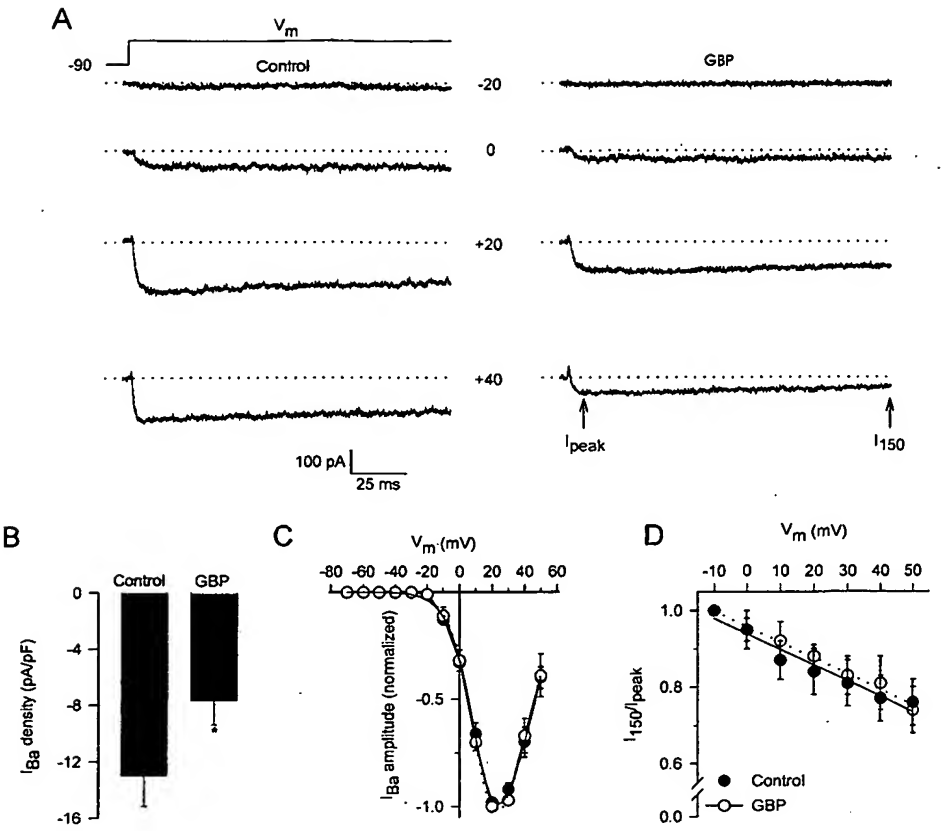

Fig. 1. Regulation of HVA Ca^{2+} channels in PC12 cells by chronic treatment with GBP. (A) Ba^{2+} currents recorded in control cells (left) and cells exposed to 30 μM of GBP for 3 days (right). GBP was not present during the patch clamping procedure. Shown are typical whole-cell recordings during 150 ms depolarizing steps from holding potential of −90 mV to the indicated voltages. (B) Comparison of current density at +20 mV in control and GBP treated cells. (C) Normalized current–voltage (I–V) relationships in control (filled circles) and treated cells (open circles). Fits of the I–V curves were obtained assuming an activation curve of a Boltzmann type. (D) The percentage of current remaining 150 ms into the depolarizing pulse is plotted at various membrane potentials in control and treated cells. Data are given as mean ± SE. The same cells were used in (B), (C), and (D), (n = 5–8 cells). Asterisk denotes significant differences ($p < 0.05$).

Table I. Biophysical Properties of Whole-Cell Ba^{2+} Currents in PC12 Cells

Kinetic properties	Control	GBP
Potential for half-activation[a] (mV)	12.93	12.49
Decay of the current at 150 ms (%)	84 ± 6	88 ± 3
Time constant of activation at +20 mV (ms)	2.08 ± 0.3	2.1 ± 0.3
Time constant of deactivation at +20 mV[b] (ms)	0.29 ± 0.01	0.26 ± 0.03

[a] Currents were recorded during 150 ms depolarizations from −90 to +20 mV.
[b] Deactivating tail currents were evoked by 50 ms depolarizations from −90 to +20 mV and fitted by monoexponential functions.

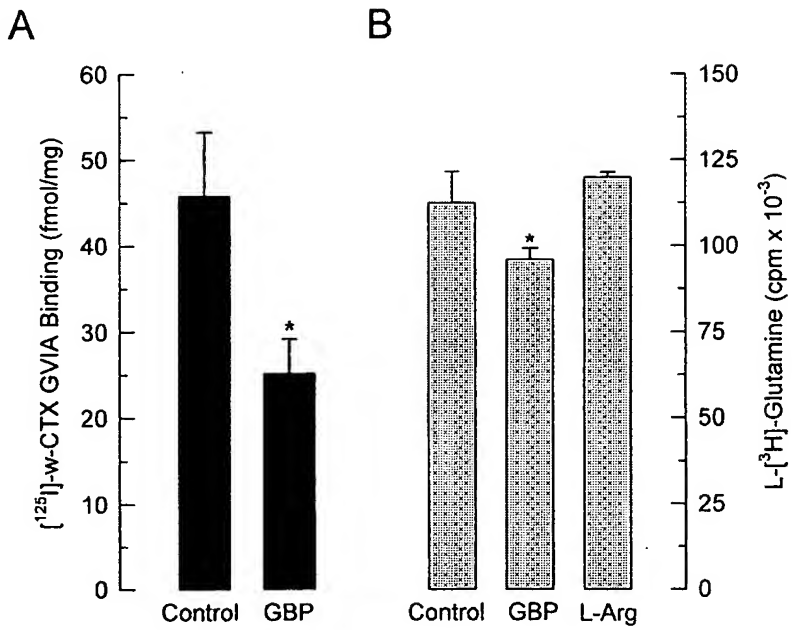

Fig. 2. Inhibition of ω-CTX and Gln specific binding by GBP. (A) Comparison of $[^{125}I]$-ω-CTX binding to microsomes of PC12 cells in control and GBP chronically treated cells. Bars represent the combined data from two separate experiments each performed by duplicate. (B) Inhibition of $[^3H]$-L Gln binding to PC12 microsomes. Excess (100 μM) of nonradioactive GBP and L-Arg was added and the amount of radioactive Gln was determined. Data are from three separate experiments in which each determination was carried out by triplicate. Asterisks denote significant differences ($p < 0.05$).

GBP but not by L-Arginine, an amino acid that normally does not utilize this transport system, suggesting a competitive interaction between GBP and the binding amino acid.

Taken as a whole, our results suggest a long-term down-regulation in the membrane expression of functional N-type Ca^{2+} channels, which might play a role in the antiepileptic action of GBP.

ACKNOWLEDGMENTS

This work was supported by CONACyT and TWAS (Grants Nos. 31735-N and 01-263RG/BIO/LA, respectively). We thank Dr Kevin P. Campbell (University of Iowa) because the initial experiments were performed in his laboratory.

REFERENCES

Alden, K. J., and Garcia, J. (2001). Differential effect of gabapentin on neuronal and muscle calcium currents. *J. Pharmacol. Exp. Ther.* **297**:727–735.

Felix, R. (1999). Voltage-dependent Ca^{2+} channel $\alpha_2\delta$ auxiliary subunit: Structure, function and regulation. *Receptors Channels* **6**:351–362.

Gee, N. S., Brown, J. P., Dissanayake, V. U. K., Offord, J., Thurlow, R., and Woodruff, G. N. (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. *J. Biol. Chem.* **271**:5768–5776.

Gurnett, C. A., De Waard, M., and Campbell, K. P. (1996). Dual function of the voltage-dependent Ca^{2+} channel $\alpha_2\delta$ subunit in current stimulation and subunit interaction. *Neuron* **16**:431–440.

Liu, H., Felix, R., Gurnett, C. A., De Waard, M., Witcher, D. R., and Campbell, K. P. (1996). Expression and subunit interaction of voltage-dependent Ca^{2+} channels in PC12 cells. *J. Neurosci.* **16**:7557–7565.

Marais, E., Klugbauer, N., and Hofmann, F. (2001). Calcium channel $\alpha_2\delta$ subunits-structure and Gabapentin binding. *Mol. Pharmacol.* **59**:1243–1248.

McLean, M. J. (1995). Gabapentin. *Epilepsia* **36**(Suppl. 2):S73–S86.

Rock, D. M., Kelly, K. C., and Macdonald, R. L. (1993). Gabapentin actions on ligand- and voltage-gated responses in culture rodent neurons. *Epilepsy Res.* **16**:89–98.

Stefani, A., Spadoni, F., and Bernardi, G. (1998). Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology* **37**:83–91.

Stefani, A., Spadoni, F., Giacomini, P., Lavaron, F., and Bernardi, G. (2001). The effects of gabapentin on different ligand- and voltage-gated currents in isolated cortical neurons. *Epilepsy Res.* **43**:239–248.

Sutton, K. G., Martin, D. J., Pinnock, R. D., Lee, K., and Scott, R. H. (2002). Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. *Br. J. Pharmacol.* **135**:257–265.

Taylor, C. P. (1993). The anticonvulsant lamotrigine blocks sodium currents from cloned alpha-subunits of rat brain Na^+ channels in a voltage-dependent manner but gabapentin does not. *Soc. Neurosci. Abst.* **19**:1631.

Taylor, C. P., Gee, N. S., Su, T. Z., Kocsis, J. D., Welty, D. F., Brown, J. P., Dooley, D. J., Boden, P., and Singh, L. (1998). A summary of mechanistic hypotheses of gabapentin pharmacology. *Epilepsy Res.* **29**:233–249.

Usowicz, M. M., Porzig, H., Becker, C., and Reuter, H. (1990). Differential expression by nerve growth factor of two types of Ca^{2+} channels in rat phaeochromocytoma cell lines. *J. Physiol.* **426**:95–116.

Wamil, A. W., and McLean, M. J. (1994). Limitation by gabapentin of high-frequency action potential firing by mouse central neurons in cell culture. *Epilepsy Res.* **17**:1–11.

Wang, M., Offord, J., Oxender, D. L., and Su, T. Z. (1999). Structural requirement of the calcium-channel subunit $\alpha_2\delta$ for gabapentin binding. *Biochem. J.* **342**:313–320.

Xie, X., Dale, T. J., John, V. H., Cater, H. L., Peakman, T. C., and Clare, J. J. (2001). Electrophysiological and pharmacological properties of the human brain type IIA Na^+ channel expressed in a stable mammalian cell line. *Pflugers Arch.* **441**:425–433.